A2 Cntd

the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110 on August 29, 2000 and assigned ATCC Accession No. PTA 2431.

IN THE CLAIMS

Please cancel Claims 1-24 and replace with the following:

A3

25. (New) A method of modifying a lysosomal hydrolase comprising contacting said lysosomal hydrolases with an isolated N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 10^6 pmol/h/mg to produce a modified lysosomal hydrolase.

Sub B2 Cont'd

26. (New) The method of Claim 25, further comprising purifying said modified lysosomal hydrolase after said contacting.

27. (New) The method of Claim 25, wherein said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

28. (New) The method of Claim 25, wherein said lysosomal hydrolase is a recombinant hydrolase.

29. (New) The method of Claim 25, wherein said lysosomal hydrolase is selected from the group consisting of α-glucosidase, α-iduronidase, α-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, β-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-α-glucosaminidase, Acetyl CoA-α-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β-galactosidase G_{M1} Galglioside, Acid β-galactosidase,

Hexosaminidase A, Hexosaminidase B, α-fucosidase, α-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β-Glucosidase.

30. (New) The method of Claim 25, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

31. (New) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 5 x 10^6 pmol/h/mg.

32. (New) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 12 x 10^6 pmol/h/mg.

33. (New) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 296 to 941 of SEQ ID NO:5.

34. (New) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions to the complement

of nucleotides 296 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

35. (New) The method of Claim 25, wherein the lysosomal hydrolase is α-glucosidase.

36. (New) The method of Claim 25, wherein the lysosomal hydrolase is α-iduronidase.

37. (New) The method of Claim 25, wherein the lysosomal hydrolase is α-galactosidase A.

38. (New) A modified lysosomal hydrolase produced by the method of Claim 25.

39. (New) A method of preparing a phosphorylated lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase, and wherein said lysosomal hydrolase comprises a N-acetylglucosamine phosphomannose diester.

40. (New) The method of Claim 39, wherein said method further comprises purifying the phosphorylated lysosomal hydrolase.

41. (New) The method of Claim 39, wherein said lysosomal hydrolase is selected from the group consisting of α-glucosidase, α-iduronidase, α-galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, β-galactosidase, iduronate 2-sulfatase, ceramidase,

galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-α-glucosaminidase, Acetyl CoA-α-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β-galactosidase G_{M1} Galglioside, Acid β-galactosidase, Hexosaminidase A, Hexosaminidase B, α-fucosidase, α-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β-Glucosidase.

42. (New) The method of Claim 39, wherein said N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from N-acetylglucosamine phosphomannose diester to generate a terminal mannose 6-phosphate on said lysosomal hydrolase.

43. (New) The method of Claim 39, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 5 x 10^6 pmol/h/mg.

44. (New) The method of Claim 39, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 12 x 10^6 pmol/h/mg.

45. (New) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

46. (New) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

47 (New) The method of Claim 39, wherein the lysosomal hydrolase is α-glucosidase.

48. (New) The method of Claim 39, wherein the lysosomal hydrolase is α-iduronidase.

49. (New) The method of Claim 39, wherein the lysosomal hydrolase is α-galactosidase A.

50. (New) A method of preparing a phosphorylated lysosomal hydrolase comprising:

contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-phosphotransferase, which has a specific activity of at least 10^6 pmol/h/mg to produce a modified lysosomal hydrolase; and

contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

51. (New) The method of Claim 50, further comprising purifying said phosphorylated lysosomal hydrolase after said contacting with the isolated N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase.

52. (New) The method of Claim 50, further comprising purifying said modified lysosomal hydrolase prior to said contacting with the isolated N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase.

53. The method of Claim 50, wherein said lysosomal hydrolase is selected from the group consisting of α-glucosidase, α-iduronidase, α-galactosidase A, arylsulfatase, N-

acetylgalactosamine-6-sulfatase, β-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-α-glucosaminidase, Acetyl CoA-α-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β-galactosidase G_{MI} Galglioside, Acid β-galactosidase, Hexosaminidase A, Hexosaminidase B, α-fucosidase, α-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β-Glucosidase.

54. (New) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 5 x 10^6 pmol/h/mg.

55. (New) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 12 x 10^6 pmol/h/mg.

56. (New) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 6% bis-phosphorylated oligosaccharides.

57. (New) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 100% bis-phosphorylated oligosaccharides.

58. (New) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 5 mannose 6-phosphates.

59. (New) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising nucleotides 133 to 3627 of SEQ

ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 296 to 941 of SEQ ID NO:5.

60. (New) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

61. (New) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 133 to 3627 of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 296 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

62. (New) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester α-N-Acetylglucosamindase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

63. (New) The method of Claim 50, wherein the lysosomal hydrolase is α-glucosidase.

64. (New) The method of Claim 50, wherein the lysosomal hydrolase is α-iduronidase.

65. (New) The method of Claim 50, wherein the lysosomal hydrolase is α-galactosidase A.

66. (New) A phosphorylated lysosomal hydrolase produced by the method of Claim 50.

REMARKS

Claims 25-66 are active in this application. Support for these claims is found in Claims 1-25 and the specification as originally filed. No new matter is added by these amendments.

The Office has restricted this application as follows under 35 U.S.C. §121:

I. Claims 1-5, 7-8, and 10-11;

II. Claims 1-5 and 9;

III. Claim 6;

IV. Claim 12-18, 20-21 and 23-24;

V. Claims 12-18 and 22; and

VI. Claim 19.

The Applicant has elected Group I, with traverse. Applicant further notes that Claims 1-24 have been replaced with Claims 25-66.

An action on the merits and allowance of the claims is requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Richard L. Chinn, Ph.D.
Registration No. 34,305

Daniel J. Pereira, Ph.D.
Registration No. 45,518

22850

(703) 413-3000
Fax #: (703) 413-2220
NFO/DJP/law

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IN THE SPECIFICATION

Please amend the specification as follows:

Page 14, line 17 to page 15, line 17, please replace the paragraph with the following paragraph:

To obtain isolated and purified GlcNAc-phosphotransferase and its subunits and the nucleic acid molecules encoding the enzyme according to the present invention, bovine GlcNAc phosphotransferase was obtained and analyzed as follows. Splenocytes from mice immunized with a partially purified preparation of bovine GlcNAc-phosphotransferase were fused with myeloma cells to generate a panel of hybridomas. Hybridomas secreting monoclonal antibodies specific for GlcNAc-phosphotransferase were identified by immunocapture assay. In this assay, antibodies which could capture GlcNAc-phosphotransferase from a crude source were identified by assay of immunoprecipitates with a specific GlcNAc-phosphotransferase enzymatic assay. Hybridomas were subcloned twice, antibody produced in ascites culture, coupled to a solid support and evaluated for immunoaffinity chromatography. Monoclonal PT18-Emphaze was found to allow a single step purification of GlcNAc-phosphotransferase to homogeneity. Bao, *et.al.*, The Journal of Biological Chemistry, Vol. 271, Number 49, Issue of December 6, 1996, pp. 31437-31445 relates to a method for the purification of bovine UDP-N-acetylglucosamine:Lysosomal-enzyme N-Acetylglucosamine-l-phosphotransferase and proposes a hypothetical subunit structure for the protein. Bao, *et. al., The Journal of Biological Chemistry,* Vol. 271,

Number 49, Issue of December 6, 1996, pp. 31446-31451. Using this technique, the enzyme was purified 488,000-fold in 29% yield. The eluted GlcNAc-phosphotransferase has a specific activity of $>10^6$, preferably $>5x10^6$, more preferably $>12x10^6$ pmol/h/mg and is apparently a homogenous, multi-subunit enzyme based on silver-stained SDS-PAGE. The monoclonal antibody labeled PT18 was selected for use in further experiments. A hybridoma secreting monoclonal antibody PT 18 was deposited with the American Type Culture Collection, 10801 Univerisity Blvd., Manassas, VA 20110 on August 29, 2000 and assigned ATCC Accession No. [_____] PTA 2432.

Page 21, line 2 to page 22, line 1, please replace the paragraph with the following paragraph:

Following a second intravenous boost with phosphodiester α-GlcNAcase, the spleen was removed and splenocytes fused with SP2/0 myeloma cells according to our modifications (Bag, M., Booth J. L., *et al.* (1996). "Bovine UDP-N-acetylglucosamine: lysosomal enzyme N-acetylglucosamine-1-phosphotransferase. I. Purification and subunit structure." *Journal of Biological Chemistry* 271: 31437 - 31445) of standard techniques; Harlow, E. and Lane, D. (1988). *Antibodies: a laboratory manual,* Cold Spring Harbor Laboratory). The fusion was plated in eight 96-well plates in media supplemented with recombinant human IL-6 (Bazin, R. and Lemieux, R. (1989). "Increased proportion of B cell hybridomas secreting monoclonal antibodies of desired specificity in cultures containing macrophage-derived hybridoma growth factor (IL-6)." *Journal of Immunological Methods* 116: 245 - 249) and grown until hybridomas were just visible. Forty-eight pools of 16-wells were constructed and assayed for antiphosphodiester α-GlcNAcase activity using the capture

assay. Four pools were positive. Subpools of 4-wells were then constructed from the wells present in the positive 16-well pools. Three of the four 16-well pools contained a single 4-well pool with anti-phosphodiester α-GlcNAcase activity. The 4 single wells making up the 4-well pools were then assayed individually identifying the well containing the anti-phosphodiester α-GlcNAcase secreting hybridomas. Using the capture assay, each hybridoma was subcloned twice and antibody prepared by ascites culture. Monoclonals UC2 and UC3 were found to be low affinity antibodies. UC1, a high affinity IgG monoclonal antibody, was prepared by ascites culture and immobilized on Emphaze for purification of phosphodiester α-GlcNAcase. The monoclonal antibody labeled UC1 was selected for use in further experiments. A hybridoma secreting monoclonal antibody UC1 was deposited with the American Type Culture Collection, 10801 Univerisity Blvd., Manassas, VA 20110 on August 29, 2000 and assigned ATCC Accession No. [_____] PTA 2431.

IN THE CLAIMS

Claims 1-24 are Canceled.

Claims 25-65 are New